PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 47/48 | A2

(11) International Publication Number: WO 97/20579

(43) International Publication Date: 12 June 1997 (12.06.97)

(21) International Application Number: PCT/EP96/05477

(22) International Filing Date: 2 December 1996 (02.12.96)

(30) Priority Data:
9524807.6 5 December 1995 (05.12.95) GB

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: PRICE, Jack [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB).

(74) Agent: FLORENCE, Julia; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*Without international search report and to be republished upon receipt of that report.*

(54) Title: NOVEL COMPOUNDS AND USE

(57) Abstract

Use of neurotoxins or fragments thereof to transport nucleic acids into the nervous system and conjugates comprising a neurotoxin or a fragment thereof and a nucleic acid.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| AM | Armenia | GB | United Kingdom | MW | Malawi |
| AT | Austria | GE | Georgia | MX | Mexico |
| AU | Australia | GN | Guinea | NE | Niger |
| BB | Barbados | GR | Greece | NL | Netherlands |
| BE | Belgium | HU | Hungary | NO | Norway |
| BF | Burkina Faso | IE | Ireland | NZ | New Zealand |
| BG | Bulgaria | IT | Italy | PL | Poland |
| BJ | Benin | JP | Japan | PT | Portugal |
| BR | Brazil | KE | Kenya | RO | Romania |
| BY | Belarus | KG | Kyrgystan | RU | Russian Federation |
| CA | Canada | KP | Democratic People's Republic of Korea | SD | Sudan |
| CF | Central African Republic | | | SE | Sweden |
| CG | Congo | KR | Republic of Korea | SG | Singapore |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LR | Liberia | SZ | Swaziland |
| CS | Czechoslovakia | LT | Lithuania | TD | Chad |
| CZ | Czech Republic | LU | Luxembourg | TG | Togo |
| DE | Germany | LV | Latvia | TJ | Tajikistan |
| DK | Denmark | MC | Monaco | TT | Trinidad and Tobago |
| EE | Estonia | MD | Republic of Moldova | UA | Ukraine |
| ES | Spain | MG | Madagascar | UG | Uganda |
| FI | Finland | ML | Mali | US | United States of America |
| FR | France | MN | Mongolia | UZ | Uzbekistan |
| GA | Gabon | MR | Mauritania | VN | Viet Nam |

# NOVEL COMPOUNDS AND USE

The present invention relates to novel therapeutic conjugates and their use in targeting therapeutic substances, in particular nucleic acids, to the central nervous system.

Large molecules introduced into the body systemically or into peripheral tissues are not transported into the nervous system in therapeutically significant amounts as they are unable to cross the so-called 'blood brain barrier'. Hence the direct therapeutic application of large molecules such as nucleic acids is currently not feasible.

Some neurotoxins have specific properties that allow them to invade the vertebrate nervous system. They bind specifically to the surface of neurons, which are the primary cellular elements of the nervous system. The neurotoxin is internalised, and retrogradely transported by those neurons to the neuronal cell body. The neurotoxin may also be transported transneuronally to second order neurons in the nervous system. In some cases it has been shown that the properties which allow transport of the neurotoxin are conferred by a particular fragment of the toxin. Examples of such toxins are clostridial neurotoxins such as those from Tetanus and Botulinum, and snake toxins such as crotoxin and dendrotoxin, from the rattlesnake and mamba snake respectively.

The transport properties of neurotoxins have been utilized to target proteins to the nervous system, by conjugating or otherwise linking the toxin or toxin fragment to such molecules, for example the enzyme horse radish peroxidase (HRP) (PS Fishman et al., J. Neurol. Sci., 1990, 98: 311-325), and wheat germ agglutinin.

In one aspect the present invention relates to the use of neurotoxins to transport therapeutically active nucleic acids across the blood brain barrier into the nervous system.

In a further aspect the present invention provides a novel conjugate comprising a neurotoxin or a fragment thereof and a nucleic acid. The conjugate of the invention may also be referred to herein as the compound of the invention.

The neurotoxins employed in the present invention may be any suitable neurotoxin which has the ability to cross the blood-brain barrier. Thus for example the neurotoxin may be derived from bacteria, such as Tetanus or Bolulinum toxin; or from snakes, such as crotoxin or dendrotoxin; or it may be a fragment of such toxins. A neurotoxin fragment can be prepared by methods well known in the protein art, for example by proteolytic cleavage or by genetic engineering strategies. Said fragment is preferably a non-toxic binding fragment.

The nucleic acids may be single or double stranded DNA or RNA molecules, either circular or linear in form, encoding either whole genes, cDNAs, non-coding sequence, genetic control regions, or antisense constructs. The nucleic acid preferably exerts a therapeutic effect.

The conjugation may be chemical in nature using chemical linkers such as poly-lysine, or covalent linkers. Other protein, nucleic acid, or other molecular components may also be part of the total conjugate, so as to endow the conjugate with other properties. For example, other components such as haemoglutinin might be included that aid trasport from the lysosomal compartment, or nuclear localisation sequences might aid transport into the nucleus. Conjugates according to the present invention may be prepared by conventional methods known in the art.

Such conjugates may be introduced into either the somatic (i.e. non-neural) or neural tissue of patients using methods known in the art, typically by hypodermic injection. By the specific binding, internalisation, and retrograde transport of the conjugate into the nervous system, the conjugate will exert a therapeutic effect.

The invention therefore further provides a pharmaceutical composition comprising a conjugate of the invention and a pharmaceutically acceptable carrier.

In use the conjugate will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The conjugate may, for example, be employed in the form of an aerosol or nebulisable solution for inhalation or a sterile solution for parenteral administration, intra-articular administration or intra-cranial administration.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired therapeutic effect. It will be appreciated that the dosage range required depends on the choice of nucleic acid, the precise nature of the conjugate, the route of administration, the nature of the formulation, the age of the patient, the nature, extent or severity of the patient's condition, contraindications, if any, and the judgment of the attending physician. Suitable daily dosages are in the range 0.01-10mg/kg, eg 0.01-5mg/kg, more particularly 0.02-1.5mg/kg, eg 0.04- 1.5mg/kg. The unit dosage can vary from less than 1mg to 300mg, but typically will be in the region of 1 to 100mg eg 1 to 50 mg per dose, which may be administered in one or more doses, such as one to six doses per day, Wide variations in the required dosage, however, are to be expected in view of the variety of nucleic acids available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition to facilitate uniform distribution of the compound.

Compositions suitable for administration via the respiratory tract include aerosols, nebulisable solutions or microfine powders for insufflation. In the latter case, particle size of less than 50 microns, especially less than 10 microns, is preferred. Such compositions may be made up in a conventional manner and employed in conjunction with conventional administration devices.

In a further aspect there is provided a method of treating a condition or disease which is susceptible of treatment with a nucleic acid in a mammal e.g. a human which comprises administering to the sufferer an effective, non-toxic amount of a compound of the invention. A condition or disease which is susceptible of treatment with a nucleic acid may be for example a condition or disease which may be treated by or requiring gene therapy.

The invention further provides a compound of the invention for use as an active therapeutic substance, in particular for use in treating a condition or disease which is

susceptible of treatment with a nucleic acid eg a condition or disease requiring or treatable by gene therapy.

The invention also provides the use of a compound of the invention in the manufacture of a medicament for treating a condition or disease which is susceptible of treatment with a nucleic acid eg a condition or disease requiring or treatable by gene therapy.

In a further aspect the invention also provides the use of a conjugate according to the present invention for the manufacture of a medicament for transporting nucleic acids to the central nervous system.

The invention also provides a therapeutic delivery system comprising a neurotoxin or a fragment thereof and a nucleic acid.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

CLAIMS

1. Use of a neurotoxin or a fragment thereof to transport a nucleic acid into the nervous system.

2. Use according to claim 1 wherein the nucleic acid exerts a therapeutic effect.

3. Use accoding to claim 1 or claim 2 wherein the neurotoxin is selected from a bacterial or snake toxin or a fragment thereof.

4. Use acording to any of claims 1 to 3 wherein the neurotoxin fragment is a non-toxic binding fragment.

5. A conjugate comprising a neurotoxin or a fragment thereof and a nucleic acid.

6. A conjugate according to claim 5 wherein the neurotoxin is selected from a bacterial or snake toxin or a fragment thereof.

7. A conjugate according to claim 5 or 6 wherein the neurotoxin fragment is obtained by proteolytic cleavage.

8. A conjugate according to claim 5 or 6 wherein the neurotoxin fragment is obtained by a recombinant method.

9. A conjugate according to any of claims 5 to 8 wherein the nucleic acid is a single or double stranded DNA or RNA molecule.

10. A conjugate according to claim 9 wherein the nucleic acid encodes a whole gene.

11. A conjugate according to claim 9 wherein the nucleic acid encodes a cDNA.

12. A conjugate according to any of claims 5 to 11 wherein conjugation is effected by a chemical linker.

13. A conjugate according to any of claims 5 to 11 wherein conjugation is effected by a covalent linker.

14. A pharmaceutical composition comprising a conjugate according to any of claims 5 to 13 and a pharmaceutically acceptable carrier therefor.

15. A conjugate of the invention for use as an active therapeutic substance.

16. A method of treating a condition or disease which is susceptible of treatment with a nucleic acid in a mammal which method comprises administering to the sufferer an effective, non-toxic amount of a conjugate according to the invention.

17. Use of a conjugate according to the invention in the manufacture of a medicament for treating a a condition or disease which is susceptible of treatment with a nucleic acid.

18. Use of a conjugate according to the invention in the manufacture of a medicament for transporting a nucleic acid to the central nervous system.

19. A therapeutic delivery system comprising a neurotoxin or a fragment thereof and a nucleic acid.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 47/48 — A3

(11) International Publication Number: WO 97/20579

(43) International Publication Date: 12 June 1997 (12.06.97)

(21) International Application Number: PCT/EP96/05477

(22) International Filing Date: 2 December 1996 (02.12.96)

(30) Priority Data:
9524807.6 5 December 1995 (05.12.95) GB

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: PRICE, Jack [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB).

(74) Agent: FLORENCE, Julia; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

**Published**
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(88) Date of publication of the international search report:
25 September 1997 (25.09.97)

(54) Title: NOVEL COMPOUNDS AND USE

(57) Abstract

Use of neurotoxins or fragments thereof to transport nucleic acids into the nervous system and conjugates comprising a neurotoxin or a fragment thereof and a nucleic acid.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| AM | Armenia | GB | United Kingdom | MW | Malawi |
| AT | Austria | GE | Georgia | MX | Mexico |
| AU | Australia | GN | Guinea | NE | Niger |
| BB | Barbados | GR | Greece | NL | Netherlands |
| BE | Belgium | HU | Hungary | NO | Norway |
| BF | Burkina Faso | IE | Ireland | NZ | New Zealand |
| BG | Bulgaria | IT | Italy | PL | Poland |
| BJ | Benin | JP | Japan | PT | Portugal |
| BR | Brazil | KE | Kenya | RO | Romania |
| BY | Belarus | KG | Kyrgystan | RU | Russian Federation |
| CA | Canada | KP | Democratic People's Republic of Korea | SD | Sudan |
| CF | Central African Republic | | | SE | Sweden |
| CG | Congo | KR | Republic of Korea | SG | Singapore |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LR | Liberia | SZ | Swaziland |
| CS | Czechoslovakia | LT | Lithuania | TD | Chad |
| CZ | Czech Republic | LU | Luxembourg | TG | Togo |
| DE | Germany | LV | Latvia | TJ | Tajikistan |
| DK | Denmark | MC | Monaco | TT | Trinidad and Tobago |
| EE | Estonia | MD | Republic of Moldova | UA | Ukraine |
| ES | Spain | MG | Madagascar | UG | Uganda |
| FI | Finland | ML | Mali | US | United States of America |
| FR | France | MN | Mongolia | UZ | Uzbekistan |
| GA | Gabon | MR | Mauritania | VN | Viet Nam |

# INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/EP 96/05477

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,Y | WO 95 32738 A (ALLERGAN INC ;DOLLY JAMES OLIVER (GB); AOKI KEI ROGER (US); WHEELE) 7 December 1995<br>see page 2, line 17 - line 28<br>see page 8, line 26 - page 9, line 8<br>see page 9, line 31 - page 10, line 2; table 1<br>see page 14, line 24 - line 29 | 1-19 |
| X | see page 15, line 1 - line 30; claims 1,2,13 | 1 |

---

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 July 1997 | 08.08.97 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax ( + 31-70) 340-3016 | Berte, M |

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO 96 04001 A (MOLECULAR STRUCTURAL BIOTECHNO) 15 February 1996<br>see page 1, line 6 - line 20<br>see page 2, line 18 - line 20<br>see page 9, line 4 - line 12<br>see page 9, line 19 - line 23<br>see page 11, line 22 - line 28; claims 1,8,16,21 | 1-19 |
| X | GENE THERAPY,<br>vol. 2, no. 7, September 1995,<br>pages 498-503, XP002036157<br>GOTTSCHALK S. ET AL.: "EFFICIENT GENE DELIVERY AND EXPRESSION IN MAMMALIAN CELLS USING DNA COUPLED WITH PERFRINGOLYSIN O." | 1-7,9-19 |
| Y | see page 498, column 2, paragraph 3 - page 499, column 1, paragraph 1 | 1-19 |
| P,X | JOURNAL OF BIOLOGICAL CHEMISTRY (MICROFILMS),<br>vol. 18, 3 May 1996, MD US,<br>pages 10560-10568, XP002036158<br>JESUS FOMINAYA ET AL.: "TARGET CELL-SPECIFIC DNA TRANSFER MEDIATED BY A CHIMERIC MULTIDOMAIN PROTEIN."<br>see page 10560, column 1, line 1-5 | 1-6,8-19 |
| Y | WO 91 00100 A (ANIMAL HEALTH INST ;IMP CANCER RES TECH (GB)) 10 January 1991<br>see page 11<br>see page 19, paragraph 2 | 1-19 |

# INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/EP 96/05477

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 96/ 05477

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Remark : Although claim 16 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ial Application No
PCT/EP 96/05477

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9532738 A | 07-12-95 | AU 2622295 A | 21-12-95 |
| | | CA 2191754 A | 07-12-95 |
| | | EP 0760681 A | 12-03-97 |
| WO 9604001 A | 15-02-96 | AU 3275595 A | 04-03-96 |
| WO 9100100 A | 10-01-91 | EP 0482018 A | 29-04-92 |
| | | GB 2252105 A | 29-07-92 |
| | | JP 5500657 T | 12-02-93 |

Form PCT/ISA/210 (patent family annex) (July 1992)